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Outcome in acute lymphoblastic leukemia: Influence of thiopurine methyltransferase genetic polymorphisms

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Abstract. Current treatment protocols for childhood acute lymphoblastic leukemia always include thiopurine drugs such as 6-mercaptopurine (6-MP), which is administrated daily during maintenance therapy. Once 6-MP is an inactive prodrug, it needs to be activated to thioguanine nucleotides (TGNs) to exert cytotoxicity. However, 6-MP can also be methylated by the polymorphic enzyme thiopurine *S*-methyltransferase (TPMT), thus reducing TGNs formation. In order to assess the influence of TPMT polymorphism in ALL, we have used PCR/HCSGE (Horizontal Conformation-Sensitive Gel Electrophoresis) based methods to characterise molecularly a sample of 110 children with ALL. Four distinct alleles associated with TPMT deficiency (TPMT*3A, *3C, *2 and *8) were found in heterozygous individuals representing 11.8% of the sample. We have also compared several parameters related with ALL outcome between subsamples of children homozygous or heterozygous for TPMT. In the heterozygous group, 6-MP dosages were lower and the number of whole interruptions during treatment or interruptions due to toxicity as well as the number of relapses was higher compared to the other group. Despite our results do not reach statistical significance, this study emphasizes the importance to introduce the prospective analysis of TPMT genotype, prior to any ALL treatment.

Keywords: Acute lymphoblastic leukemia; Thiopurine *S*-methyltransferase; 6-Mercaptopurine

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1. Introduction

Contemporary treatment protocols for childhood acute lymphoblastic leukemia (ALL) always include thiopurine drugs such as 6-mercaptopurine (6-MP), which is administered daily during maintenance therapy.

Once 6-MP is an inactive prodrug it requires bioactivation by a multistep pathway to form thioguanine nucleotides (TGNs), which are incorporated into DNA and RNA to exert cytotoxicity. However, this process is in competition with inactivation of mercaptopurine or its metabolites by methylation mediated by thiopurine *S*-methyltransferase (TPMT) [1].

TPMT exhibit autosomal codominant genetic polymorphisms and until now several variant alleles associated with decreased activity are known. It has been demonstrated that the therapeutic efficiency and toxicity of thiopurine drugs depends on levels of TPMT activity, so deficient methylators can be at risk of hematopoietic toxicity when treated with conventional doses.

The objective of this study was to evaluate the relationship between molecular TPMT genotype and the reaction to thiopurine drugs as well as to assess the importance of determining the TPMT genotype before initiating thiopurine therapy.

2. Material and methods

Patients enrolled in this study were 110 children submitted to acute lymphoblastic leukemia therapy in the Hospital Geral S. João or in the Portuguese Institute of Oncology, both in Oporto. The clinical histories of 61 of these patients were analyzed for several parameters. The presence of TPMT*2, TPMT*3A, TPMT*3B, TPMT*3C and TPMT*8 mutant alleles were screened using PCR/HCSGE (Horizontal Conformation-Sensitive Gel Electrophoresis), and the allele frequencies were estimated by direct gene-counting method [2].

3. Results

In the 110 children with ALL enrolled in this study seven were found to be heterozygous for the TPMT*3A allele, three for the TPMT*3C, one for TMPT*2, and two for TPMT*8, representing

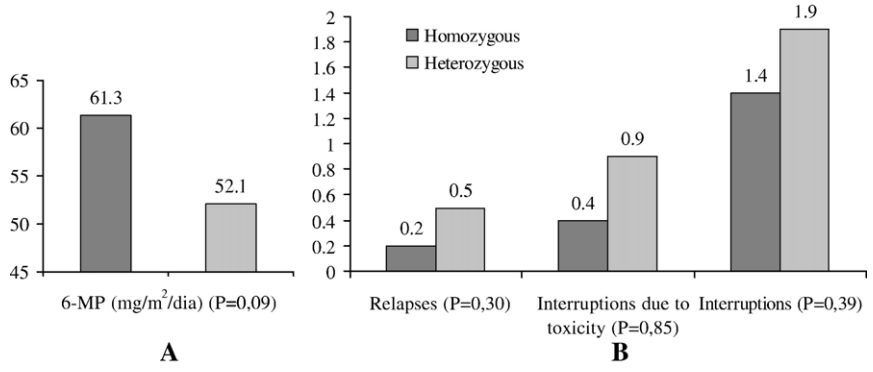

Fig. 1. Comparison of several parameters related to ALL outcome between sub-samples of homozygous and heterozygous patients; (A) doses of 6-MP administered (mg/m^2/dia); (B) average number of relapses, interruptions due to toxicity and whole treatment interruptions.

11.8% of the sample. The remaining 97 patients were homozygous for the wild-type allele (TPMT*1).

Several parameters related to ALL outcome were compared (Mann–Whitney test) between the sub-samples of heterozygous ($N=10$) and homozygous ($N=51$) patients (Fig. 1).

The average number of whole treatment interruptions as well as those due to toxicity was slightly higher in the heterozygous patients than in the homozygous group. The same tendency was observed with concern to relapses. The most accentuated difference occurred in 6-MP doses administered to each group, once heterozygous patients received lower doses comparing with "wild-type" patients. However, differences between the two groups did not reach statistical significance.

4. Discussion

The combined frequency of all alleles associated with enzymatic deficiency (0.06) was not significantly different ($P=0.67$) from that reported for Northern Portuguese population, indicating that TPMT polymorphism is not a factor of ALL susceptibility [2].

It has been demonstrated that the therapeutic efficiency and toxicity of thiopurine drugs is largely dependent on levels of TPMT activity. In this study we observed that heterozygous patients usually need to interrupt 6-MP administration and relapses more frequently than homozygous patients. Consequently, patients with intermediate TPMT activity received lower doses of that drug. The absence of statistically significant differences between groups might be due to the still small sample sizes, particularly to the low number of heterozygous patients. There are other factors that may also influence the response to treatment with 6-MP, and thus the therapy success, which were not considered in our study. First, other chemotherapeutic agents, given concomitantly to 6-MP (such as methotrexate), may also contribute to toxicity events [3]. Moreover, polymorphisms affecting the activity of other enzymes involved in the metabolism of thiopurine drugs may also influence ALL outcome [4]. In future studies these parameters should be considered.

Since TPMT represents a determinant of 6-MP response and ALL outcome, this study reinforces the relevance to introduce the prospective analysis of TPMT prior to any treatment, in order to individually optimize 6-MP therapy and avoid adverse reactions to this drug.

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